UDC 541.64

*Makhayeva D., Irmukhametova G., Mun G.

Al-Farabi Kazakh National University, Almaty, Kazakhstan
*e-mail: danelya_1993@mail.ru

Mucoadhesive properties study of films based on N-vinylpirrolidone-2-hydroxymethylacrylate and acrylic acid copolymers

Abstract: The films based on N-vinylpirrolidone and 2-hydroxymethylacrylate (NVP-HEMA) copolymers and N-vinylpirrolidone-2-hydroxymethylacrylate and acrylic acid (NVP-HEMA-AA) triple copolymers were synthesized by free radical copolymerization. Complex formation of linear NVP-HEMA and NVP-HEMA-AA with mucin was studied. It has been found that the complex formation is a result of specific hydrogen bonds and van der Waals interactions. Also the pH of complexation for binary and triple copolymers system is around 5.5-6.5. The investigation of mucoadhesive properties of films based on linear copolymers was carried out and the impact of their composition on retention times of films on mucosa surface of pork buccal was istablished. The presence of HEMA and AA in copolymers composition increases the adhesive properties of films. Thermal treatment of polymeric films based on the NVP, HEMA and AA in order to get crosslinked structure resulted in lowering their mucoadhesive properties. Thus, it is found that the retention time of the films on mucosal surfaces of the pork oral cavity primarily depends on the composition of copolymers and a the way of films preparation.
Key words: mucoadhesion, hydroxymethylacrylate, N-vinylpirrolidone, acrylic acid.

Introduction

At the beginning of the XXI century there was a popular trend toward scientists developing a new mucoadhesive dosage forms. The main positive property of these delivery systems is consolidating on the mucous membranes, which is used as a way of solving the problem of low bioavailability of conventional formulations used in the oral cavity, on the surface of the eye or other parts of the body. In these parts of the body prolonged retention of drugs are prevented by movement of tissue and formation of different secrets. Application of mucoadhesive ointments based on polyacrylic acid and polymethyl methacrylate was a first theme of early investigations.

At the present time various types of synthetic and natural polymers get popular for the development of new pharmaceutical drug delivery systems. Examples of such polymers are derivatives of cellulose such as hydroxypropylcellulose (HPC), hydroxypropylmethylcellulose (HPMC), hydroxyethylcellulose (HEC), sodium carboxymethylcellulose (KMC), polyacrylic acid derivatives, polyacrylates, polycarbophyl, a copolymer of methyl vinyl ether and methacrylic acid, poly-2-hydroxyethylmethacrylate, polyvinylpyrrolidone (PVP), polyvinyl alcohol (PVA), chitosan, xanthan, pectin and alginate.

When polymers are moistered, they have viscous forms, which increase their residence time on the mucosal surface and result in adhesive interactions [1]. Adhesive interactions include hydrogenous bonds, hydrophobic interactions, van-der-Waals interactions, electrostatic coupling, covalent bonds [2,3]. For these reasons, high-molecular compounds with a high content of polar groups such as COOH and OH, characterized by a stronger mucoadhesion with minimal exposure of toxins [2].

Mucoadhesive properties depend on molecular weight of polymers, their flexibility, degree of hydration, hydrophilic-hydrophobic balance and the presence of crosslinking units in the macromolecules inspite of influence various functional groups in a polymer chain [4-7]. At present paper the polymeric films were formed from new potentially mucoadhesive copolymers based on NVP, HEMA and AA and their ability to retain on mucosa surface was studied.

Materials and methods

Materials

N-vinylpirrolidone, 2-hydroxymethylacrylate and mucin from porcine stomach type II were purchased from Sigma Aldrich (Great Britain) and used without purification. Acrilic acid was purchased from Russia with mark «clean». Azobisisobutyronitrile was purchased from Acros (USA).

Artificial saliva solution was prepared according to the method described in paper [8]. The chemical

 Printed in Kazakhstan

composition of the artificial saliva: disodium hydrogenorthophosphate (Na_2HPO_4), sodium hydrogencarbonate ($NaHCO_3$), calcium chloride ($CaCl_2$), distilled water (H_2O) and a solution of 1M hydrochloric acid (HCl).

Synthesis of copolymers based on NVP, HEMA and AA.

The copolymers of different composition were synthesized by free radical copolymerization method. Synthesis was carried out in glass ampoules with alcohol as a solvent and azobisisobutyronitrile as an initiator at temperature of 60°C (333 K). The ratio monomer mixture: solvent was 30:70 vol. %. For oxygen expulsion the reaction mixture was blown by argon during 15 minutes. Synthesised copolymers were purified from unreacted monomers by multireprecipitation from an ethanol solution to ethyl acetate. After that copolymers were dried in vacuum oven for a few days till constant weight.

Films preparation.

The aqueous copolymer solutionts with concentrations of 0.1, 0.3 and 0.5 M were prepared and cast onto plastic molds. After that the samples were dried for 24 hours in the open air at room temperature to constant weight.

The film thickness was measured in five different places (center and four corners) by digital micrometer.

Crosslinked films preparation and characterisation.

Crosslinking of polymers was performed by their thermal treatment. Previously prepared films by casting to plastic molds were placed into drying oven at the three temperature regimes, such as 90°C, 110°C and 130°C for 5, 7 and 9 hours.

The swelling degree of thermo-crosslinked films was calculated using the following equation:

$$\alpha = \frac{m_{swel} - m_{dry}}{m_{dry}}$$

где, m_{swel} – weight of equilibrially swelled film; $m_{сух}$ – weight of dry film.

Interpolymer complex solutions were prepared by mixing the polymers at certain ratio for 5 minutes to reach equilibrium and measured.

Methods of analysis

IR spectra of copolymers were registered using IR spectrophotometer «Satellite FTIR Mattson» (USA) in the mid-IR area. For analysis the tablets were prepared from the powders of copolymers and calcined potassium bromide.

Turbidimetric analysis of interactions between mucin and water solutions of copolymers was carried out using UV-spectrophotometer «Shimadzu UV/VIS-2401 PC» under the following conditions: T= 25^{0}C (298K) and λ=400 nm. To maintain the appropriate temperature the cuvettes with thermostatic regulated cell were used.

Rheological method of copolymers mucoadhesive properties evaluation was carried out using microviscometer «Lovis 2000 M/ME AntonPaar» (Austria).

Sizes of copolymers and mucin-copolymer complexes was determined at 25°C by dynamic light scattering on «Malvern Zetasizer Nano-S» (Malvern Instruments, Great Britain).

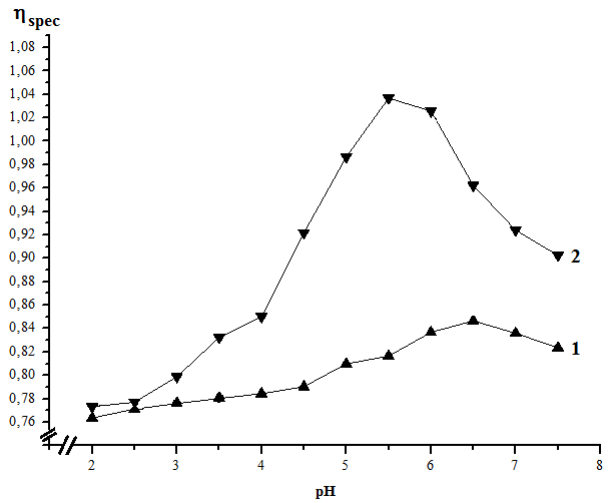
Mucoadhesive properties of films was studied by fixing their sample to a freshly prepared mucosa of pork cheek and set on a rotating disk (rotation speed 59 rpm). After that the disc was placed in a solution of artificial saliva (pH 6,5-6,8, and T=36,5^{0}C). The time required for complete separation or dissolution of the film was determined visually.

Results and their discussion

Copolymers of different composition were synthesized with the following ratio of monomers in the initial monomer mixture (IMM) [NVP]:[HEMA] = 90:10, 80:20 and 70:30 mol% and [NVP]:[HEMA]-[AK] = 90:10-5, 80:20-5 and 70:30-5 mol%.

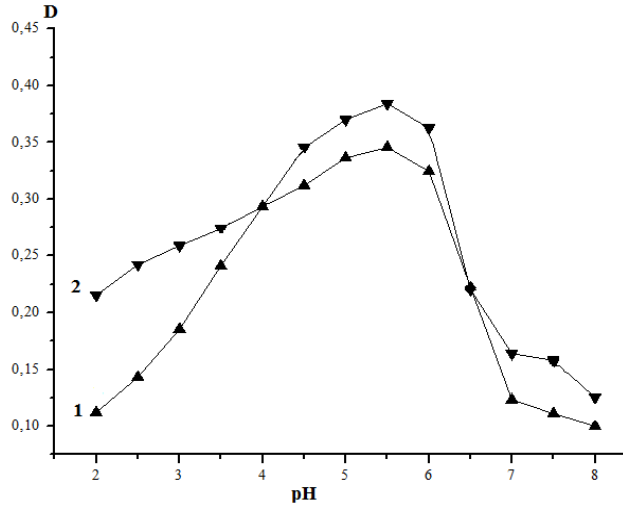
The impact of solution pH on complex formation between mucin and synthesized copolymers was studied using viscosimetric and turbidimetric titration. Graphical results of complexation study are depicted at figures 1 and 2. The critical pH value of complexation was found to be between pH 5.5-6.5. Moreover, pH of complexation for terpolymers system lies in the region closer to 5,5. The observed shift of pH complexation for tercopolymers in acidic region is due to suppression of acrylic acid carboxyl groups dissociation and electrostatic repulsion between the charged groups of mucin and copolymers.

Influence of copolymers solution concentration on their interaction with mucin molecules was studied (figure 3). Conjugated proteins of mucin have a negative charge. As a result of interaction between mucin and copolymer solutions, particles of copolymers adsorbed onto surface of conjugated proteins. And if concentration of copolymers in solution increases, there will be aggregation with mucin particles. pH of complexation descends if concentraion of polymers increases, because of growth of copolymer particles participated in complexation with mucin (figure 3).



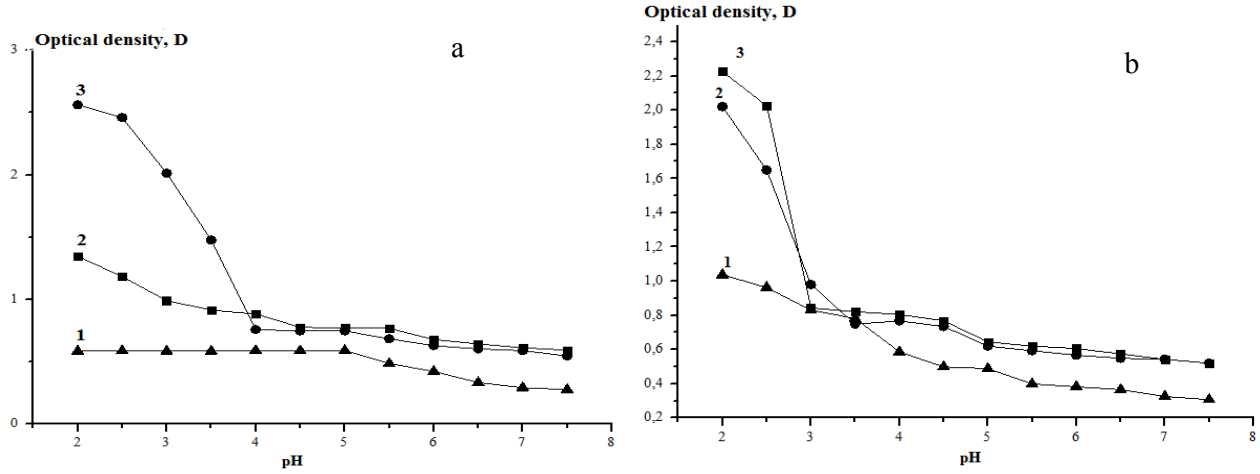
Concentration of mucin and copolymer solutions is 1 mg/mL
[NVP:HEMA] =[80:20] (1);
[NVP:HEMA]-[AA] =[80:20]-[5] mol% (2).

Figure 1 – Viscometric titration of copolymer solutions with mucin



[Concentration of mucin and copolymer solutions is 1 mg/mL
[NVP:HEMA] =[80:20] (1);
[NVP:HEMA]-[AA] =[80:20]-[5] mol% (2).

Figure 2 – Turbidimetric titration of copolymer solutions with mucin



C_{cpl} 0,003 M (1); 0,01 M (2); 0,05 M (3)
[NVP:HEMA] =[80:20] (a); [NVP:HEMA]-[AA] =[80:20]-[5] mol% (b)

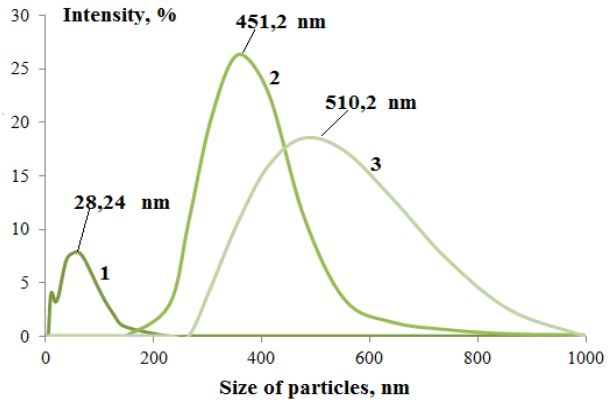
Figure 3 – Turbidimetric study of copolymer solutions concentration impact on complex formation with mucin molecules

Dynamic light scattering was used also to study the complex formation between macromolecules of copolymers and mucin. In order to confirm interaction the particle sizes of copolymers, mucine and their mixtures were determined. Results of analysis are presented in figures 4 and 5. It can be seen from the figures, that after mixing the copolymers solution with mucin the intermolecular complex formation occur which is resulting in increasing the particle size of macromolecular agglomerations.

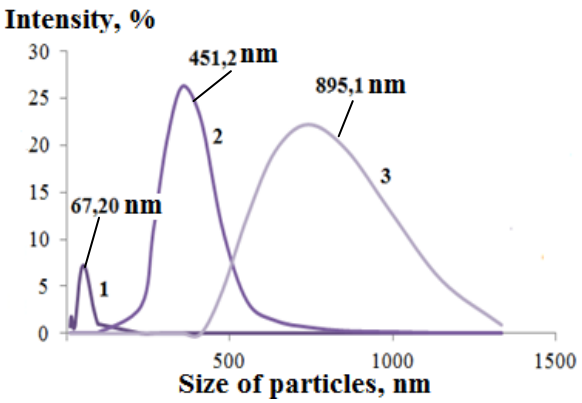
Linear and thermally crosslinked polymeric films based on NVP-HEMA and AA copolymers were casted and swelling degree of crosslinked samples was studied. Kinetics of films swelling was analysed to determine the possibility of their using as mucoadhesive materials. For this purpose a films were thermally treated at T = 90, 110 and 130 °C for 5, 7 and 9 hours. Usually, thermal treatment is performed to improve the mechanical properties of the products, reduce the residual tension, which accumulate in the

products during molding and to reduce the content of volatiles in the material.

The swelling kinetics and degree of different films was estimated in distilled water, ethanol-water solution (50:50 vol.%) and a solution of artificial saliva. Results of analysis are presented in figure 6. The content of acrylic acid chains in copolymers improves swelling ability of films in all kind of surrounding mediums. This is due to the fact that carboxylic groups of acrylic acid enhance the hydrophylic properties.



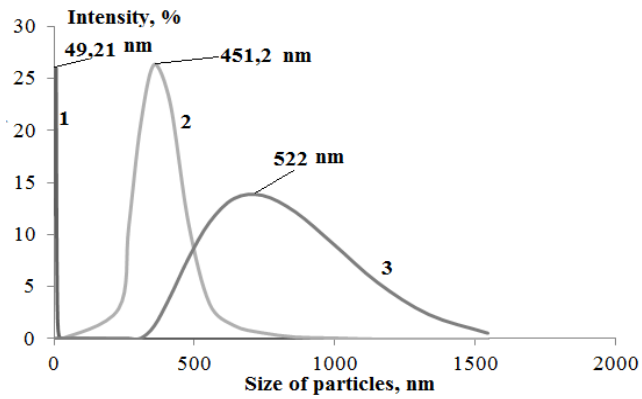
1 – [NVP:HEMA] [90:10] mol%; 2 – mucin;
3 – mixture of mucin with NVP:HEMA copolymer



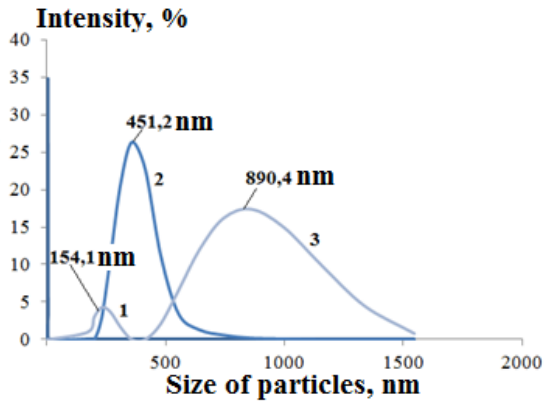
1 – [NVP:HEMA-AA] [90:10]-(5) mol%; 2 – mucine;
3 – mixture of mucin with NVP:HEMA-AA copolymer

[mucin]:[copolymer] = [1:1] by volume, T = 25°C, pH = 6.5~6.8.

Figure 4 – Graphs of particles size distribution of individual copolymer, mucin and their mixtures



1 – [NVP:HEMA] [80:20] mol%; 2 – mucin;
3 – mixture of mucin with NVP:HEMA copolymer



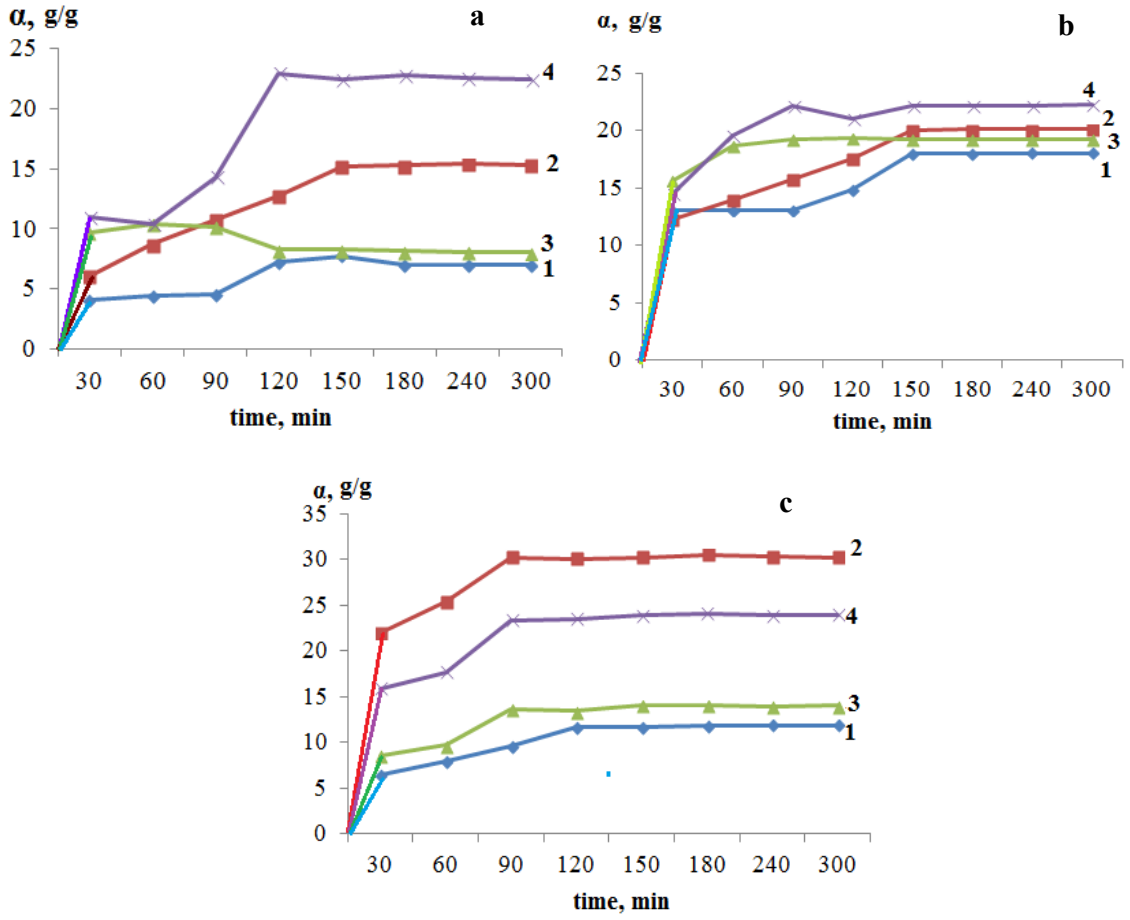
1 – [NVP:HEMA-AA] [80:20-(5%)]; 2 – mucine;
3 – mixture of mucin with NVP:HEMA-AA copolymer

[mucin]:[copolymer] = [1:1] by volume, T = 25°C, pH = 6.5~6.8.

Figure 5 – Graphs of particles size distribution of individual copolymer, mucin and their mixtures

Mucoadhesive properties of crosslinked and non-crosslinked films based on NVP, HEMA and AA copolymers were studied. Thus, a sample (film) is brought into contact with fresh pork buccal mucosa and fixed on rotating disk. The system was immersed in a solution simulating the environment of the oral cavity (pH = 6.5~6.8) at T = 35-36 °C. The retention time or dissolution time of films were determined visually. Results are represented in a table 1 and 2. For the films based on linear copolymers NVP-HEMA and NVP-HEMA-AA the presence of AA in IMM results in enhancing the mucoadhesive properties and increasing the retention time up to 5 minutes. In case of thermally crosslinked films the samples containing

AA posses lower ability to adhere to mucus surface after heating them probably because the crosslinking process involves the carboxylic groups of AA and acrylate groups of HEMA which responsible to mucoadhesion. Also increasing the heating temperature for copolymers crosslinking results in worsening the mucoadhesive properties of polymeric films.



[NVP:HEMA] =[90:10] (1); [NVP:HEMA]-[AA] =[90:10]-[5] (2);
[NVP:HEMA] =[80:20] (3); [NVP:HEMA]-[AA] =[80:20]-[5] (4) mol%
Thermal crosslinking conditions: 110 °C and 5 hours

Figure 6 – Swelling kinetics of films based of NVP-HEMA and NVP-HEMA-AA copolymers in water (a), ethanol-water solution (b) and solution of artificial saliva (c)

Table 1 – Mucoadhesive properties of non-crosslinked films based on copolymers of NVP, HEMA and AA.

IMM content of copolymers, mol%	Retention/dissolution time, min	Observation
[NVP:HEMA] =[90:10]	25	Detachment
[NVP:HEMA]-[AK] =[90:10]-[5]	30	Dissolution
[NVP:HEMA] =[80:20]	26	Detachment
[NVP:HEMA]-[AK] =[80:20]-[5]	32	Dissolution

Table 2 – Influence of crosslinking temperature on retention time of thermo-cross linked films based on copolymers of NVP, HEMA and AA

IMM content of copolymers, mol%	Crosslinking temperature		
	90°C	110°C	130°C
[NVP:HEMA] =[90:10]	30 min	10 min	5-10 min
[NVP:HEMA]-[AA] =[90:10]-[5]	20 min	5 min	5 min

Conclusion

As a result of investigation the complex formation between mucin and copolymers based on N-vinylpirrolidone, 2-hydroxymethilacrylate and acrilic acid. Impact of pH on interaction of copolymers with mucine molecules was observed. The maximum complexation was observed at interval of pH 5.5-6.5. Also the presence of interaction of copolymers with mucin was proved using DLS analysis which shows significant growth of particle size of macromolecules after mixing them. The films with linear and crosslinked structure based on copolymers were obtained and their mucoadhesive properties were studied. Retention time of films on pork bucal mucosal surface was found to be depended on composition of copolymers and temperature of thermal crosslinking of copolymers. Presence of AA increases the retention time of films but thermal treating reduces the mucoadhesive properties of the films, which is apparently due to the fact that thermal crosslinking proceeds by functional groups of AA and HEMA, which are responsible for the mucoadhesive properties of the films.

References

1. Birudaraj R., Mahalingam R. Advances in buccal drug delivery // Crit. Rev. Ther. Drug Carrier Syst.-2005. – Vol.3 (22). – P.295-330.

2. Khutoryanskiy V. Advances in Mucoadhesion and Mucoadhesive Polymers // Macromol. biosci. – 2011. – Vol. 11. – P. 748-764.

3. Abdelghany A.M., Abdelrazek E.M., Rashad D.S. Impact of in situ preparation of CdS filled PVP nano-composite // Spectrochim acta A J. – 2014. – Vol. 130. – P.302-308.

4. Sosnik Alejandro Mucoadhesive polymers in the design of nano-drug delivery systems for administration by non-parenteral routes: A review // J Polym. Sci. Pol. Chem. – 2014. – Vol.39. – P.2030-2075.

5. Müller C, Leithner K, Hauptstein S, Hintzen F, Salvenmoser W, Bernkop-Schnürch A. Preparation and characterization of mucuspenetrating papain/poly(acrylic acid) nanoparticles for oral drug delivery applications // J Nanopart. Res. – 2012. – Vol.15. – P.11–13.

6. Grabovac V, Guggi D, Bernkop-Schnürch A. Comparison of the mucoadhesive properties of various polymers// *Adv. Drug Del. Rev*.- 2005. – Vol. 57. – P.1713–1723.

7. Andres-Guerrero V., Molina-Martinez I. and others The Use of Mucoadhesive Polymers to Enhance the Hypotensive Effect of a Melatonin Analogue, 5-MCA-NAT, in Rabbit Eyes // *IOVS*. – 2011. – Vol.52. – P.1507-1515

8. Mariano N.A., Oliveira R.G., Fernandes M.A., Ringo E.C.S. Corrosion behavior of pure titanium in artificial saliva solution // Revista Materia. – 2009. – Vol.14, №2. – P.878-880.